Testicular fine needle aspiration biopsy in male infertility: A review of the indications, the advantages and the clinical applications

Themistoklis Mikos, Dimitrios G. Goulis, Paris Polichronou, Grigorios Grimbizis, John N. Bontis, John Papadimas

Unit of Reproductive Endocrinology, First Department of Obstetrics & Gynecology, Aristotle University of Thessaloniki, Papageorgiou General Hospital, Thessaloniki, Greece

ABSTRACT: Testicular Fine Needle Aspiration (FNA) biopsy is an integrated part of the diagnostic and the therapeutic approach of the infertile man. Apart from the cytological diagnosis, FNA makes possible sperm collection that can be subsequently used in Intra-Cytoplasmic Sperm Injection (ICSI). Compared to the traditional open testicular biopsy, FNA has significant advantages as it is a fast, low cost, less traumatic and minimally invasive technique. The combination of FNA with ICSI has revolutionized the management of male subfertilty. Infertile men with spermatogenesis defects can father their own children, whereas in the near past donor sperm or adoption were the only options available.

Key Words: Testicular FNA, Male infertility, Azoospermia, ICSI.

1. INTRODUCTION

Infertility is defined as failure of conception after at least twelve months of timely, unprotected intercourse. It is a common problem affecting 14-26% of the couples. Male factor is mainly responsible in 30% of the infertile couples and it is a co-factor in an additional 20%. Basic investigations for the infertile man include anamnesis, clinical examination, hormonal tests (serum FSH, LH, prolactin, total testosterone), and semen analysis (two to three samples in a six-month period). Recently, in selected cases further genetic (karyotyping and Polymerase Chain Reaction - PCR) and cytological investigations (testicular Fine Needle Aspiration biopsy - FNA) have been introduced[1].

FNA is a cytological examination that has been widely used for the diagnosis of lesions of the thyroid gland, breast, intraperitoneal organs and head and neck tumors[2,3]. FNA sets the cytological diagnosis with high sensitivity and specificity. The method is universally accepted for its minimally invasive nature: it is performed under local anesthesia, in ambulatory patients at an outpatient clinic or a day-surgery basis and it has minimal or no complications. More specifically, during testicular FNA spermatozoa can be collected and subsequently used in Assisted Reproduction Techniques (ART) such as Intra-Cytoplasmic Sperm Injection (ICSI). Apart from its therapeutic role, testicular FNA assists in the cytological - histological diagnosis of the infertile man and in the management and counseling of these couples.

In this article, we review the indications, the technique and the interpretation of testicular FNA biopsy. The method is compared to open testicular biopsy and its clinical applications in reproductive medicine are discussed.

2. Clinical indications for testicular biopsy (open or fna)

Main clinical indications of testicular biopsy are:

1. *To differentially diagnose obstructive from non-ob-*

Corresponding author: Themistoklis Mikos, Unit of Reproductive Endocrinology, 1st Department of Obstetrics & Gynecology, Aristotle University of Thessaloniki, Papageorgiou General Hospital, Periferiaki Odos Neas Efkarpias, 546 03, Thessaloniki, Greece, Tel.: +30 2310 693131, Fax: +30 2310 991510, E-mail. themis.mikos@gmail.com

structive azoospermia in men with normal or borderline testicular size, palpable ejaculatory ducts and normal Follicular Stimulating Hormone (FSH) serum levels[7]. Testicular biopsy sets the histological diagnosis, i.e. whether azoospermia is caused by primary testicular failure, it is secondary to obstruction or there is a combination of these two conditions. Therapeutic management is then appropriately selected.

2. *In cases of obstructive azoospermia with concurrent varicocele.* Normal spermatogenesis in these men confirms obstruction, whereas hypospermatogenesis or spermatogenesis arrest usually is interpreted as negative influence of the varicocele on the spermatogenesis, and therefore it is an indication for surgical correction.
3. *In cases of primary testicular failure with concurrent varicocele.* Sertoli Cell-Only Syndrome (SCOS) favors the diagnosis of primary testicular failure, whereas spermatogenesis arrest indicates that varicocele affects the spermatogenesis and therefore it should be corrected.
4. *Other special indication for testicular FNA biopsy includes the detection of spermatozoa in azoospermic men prior to ART.* Sperm detection in these cases is a good prognostic factor for sperm retrieval in subsequent open testicular biopsy (TEsticular Sperm Extraction - TESE). If no sperm is retrieved during the FNA, open biopsy is still indicated as a TESE - ICSI procedure; the counseling, however, of the couple includes the option of sperm donor use. Common causes of azoospermia are Idiopathic Non-Obstructive Azoospermia (INOA), cryptorchidism, Klinefelter syndrome, epididymal, vasal, or ejaculatory duct pathology, and Congenital Bilateral Absence of the Vasa Deferentia (CBAVD).

3. FNA technique

Obrant and Persson are considered the pioneers of testicular FNA biopsy. In 1965 they published a case-series of 42 azoospermic men investigated with this method[8].

Testicular FNA is performed under local anaesthesia. A few centers have suggested intravenous morphine for patient sedation, a variation that has not been widely accepted. The clinician carefully cleanses the scrotum and the genital area with antiseptic solution and the surgical field is then isolated with sterile drapes. The area around the seminiferous duct is infiltrated with local anesthetic. Further local anesthetic is infused in the scrotal skin at the sites of the biopsy punctures. The clinician holds the testicle with the thumb and the index finger just beneath the scrotal skin in order to avoid any epididymal damage during biopsy. The 23 G biopsy needle is then introduced in two to four different testicular sites. Biopsies are usually taken from the upper and the lower testicular pole and in multiple site biopsies protocols from the middle of the testicle. The biopsy needle (a "butterfly" one) is attached to an empty 20-ml disposable syringe, usually mounted on a syringe holder for single hand grip. During needle insertion into the testicle, the clinician withdraws the syringe pump creating a continuous negative pressure that helps biopsy material to be obtained from the testicle. The aspirates are then deposited on clean glass slides and the smears are air-dried and fixed. May-Grünwald-Giemsa or Papanicolaou staining are the common techniques.

For the last two years in the Unit of Reproductive Endocrinology of the First Department of Obstetrics & Gynecology of the Aristotle University of Thessaloniki, ThinPrep material embedding is used with results equal to the traditional fixation techniques. In a study of 15 azoospermic men where ThinPrep embedding of the material obtained from testicular FNA was compared to the traditional Papanicolaou fixation, no significantly statistical difference in the number of the cells that were isolated (first class spermatocytes, spermatids, spermatozoa and Sertoli cells) as well as in the cytological diagnosis were found. Moreover, ThinPrep made cell type recognition less difficult. This is mainly due to the improved stabilization of the biopsy material, the limited number of red cells that obscure the sample and the finer cell layer that ThinPrep compared to the conventional fixation techniques establishes[11].

4. FNA interpretation

Testicular FNA interpretation takes part in two steps. Initially, in low magnification, the cytologist assesses whether the material is adequate enough and takes an overall impression of the presence of a multiple

Table 1. Summary of studies where testicular FNA is compared to open biopsy.

	Prospective design	Number of patients	Number of testicles biopsied	Needle size (G)	Histopathological /Cytological agreement	Histopathological /Cytological agreement (%)
Gottschalk-Sabag et al. (1993)	No	55	61	23	54 / 61	88.5
Odabas et al. (1997)	N/A	29	58	23	52 / 58	89.6
Rosenlund et al. (1998)	No	10	16	19	7 / 16	43.7
Mahajan et al. (1999)	Yes	60	60	21	58 / 60	96.6
Aridogan et al. (2003)	N/A	40	76	26	69 / 76	90.7
Total		194	271			88.5

N/A = Non Available.

or single cell image. Then, in high magnification, the cells are recognized and the spermatogenesis damage, if any, is classified[12].

There are two systems of testicular FNA biopsy interpretation: the traditional classification as described by Foresta[13] and a more recent one by Meng[14]. According to Foresta, the material is initially May-Grünwald-Giemsa stained. For the interpretation of the sample, it is necessary to measure 200 consecutive cells and their percentage. Cytological diagnosis is based on the predominant type of cells:

1. *Normal spermatogenesis:* cells from all the stages of spermatogenesis are detected.
2. *Hypospermatogenesis:* although cells from all the stages of spermatogenesis are encountered, their number is significantly reduced. It should be noted that in hypospermatogenesis cases, spermatozoa detection in seminiferous tubules is achieved.
3. *Maturation arrest:* Sperm maturation stops in early stages of spermatogenesis. Nor sperms, neither spermatids are detected. The arrest is usually in the stage of first class spermatocytes.
4. *Sertoli Cell-Only Syndrome:* Spermatogenesis cells are completely absent, Sertoli and Leydig cells being the only cells detected.

According to the Meng FNA classification system, the sample is stained as per Papanicolaou and the pathologist assesses only the presence of spermatozoa, spermatids and first class spermatocytes. Compared to the Foresta, the Meng classification system recognizes two subgroups of spermatogenesis arrest: the early, which is considered always to be a complete arrest and the late, which is considered always to be an incomplete arrest.

5. Comparison between FNA and open biopsy

Testicular FNA presents a lot of significant advantages compared to the open biopsy: (a) FNA makes possible biopsy from multiple, different testicular sites, which is per se more representative, (b) histology samples from open testicular biopsy are not uncommonly complicated by artifacts, (c) FNA is swift, non-expensive, and non-traumatic method and (d) FNA is less invasive compared to the open biopsy.

Very few studies in the literature describe a comparison between histological diagnosis from material obtained by open testicular biopsy and cytological diagnosis from testicular FNA. After careful selection of the best methodology studies, the agreement between open and FNA biopsy diagnosis in 194 infertile men was 88.5% (Table 1)[18,19,20].

Testicular FNA appears to be a more reliable diagnostic and sperm retrieval method compared to the open biopsy. The clinician can take samples almost from every site of the testicles. The fine needle insertion affects a restricted only area of the testicle, compared to the more extensive tissue involvement during the open biopsy. The risk of damaging a testicular vessel during FNA is minimal. Thus, the risk of endotesticular bleeding post FNA is only 7% compared to 29% after an open testicular biopsy[21]. Other studies provide evidence that the multiple incisions at the tunica albinea performed during open biopsy cause a reduction to the testicular vascularization[22,23]

and increase the possibility of ischemic testicular damage[15].

On the other hand, testicular FNA is still not a routine technique in the investigation of the male infertility. There are several reasons for this: (a) the diagnostic reliability of FNA biopsy is challenged, especially when compared to the open biopsy, (b) there is a constant fear of possible testicular trauma and haematomata after FNA biopsy, as well as the fear of malignant cells contamination[15,24,25,26] and (c) the lack of experienced cytologists in the field of Andrology and the absence of studies that support FNA are further factors that obscure the universal use of this technique[27]. According to some studies, testicular FNA biopsy is inferior to the open biopsy for the evaluation of spermatic tubules and testicular architecture[20,21]. It is common sense, that the larger the testicular sample obtained during a biopsy, the more likely the detection of spermatic tubules with normal spermatogenesis[28]. Therefore, the diagnostic accuracy of the testicular biopsy and the number of sperm retrieved depends largely on the degree of invasiveness during the biopsy. Unfortunately, this contradicts to the attempt to keep as much of the testicular tissue as possible intact. In a recently published study, an attempt to define any differences in the histological findings post open and testicular FNA biopsy in guinea pigs was made. This study demonstrated that the damage on the testicular tubules and Sertoli cells is more extensive after FNA biopsy. The damage after FNA biopsy takes place slowly, within a month's time, causing a permanent destruction of the testicular tubules of the initial biopsy site and those of the neighboring tissue as well. After an open testicular biopsy, however, a permanent scarred tissue is developed in the biopsy site, without extension to adjacent tubules[29]. The unexpected findings of more extensive tissue damage post testicular FNA in the laboratory model should be very cautiously interpreted and not be extrapolated to humans, as there are no clinical studies indicating such effect of FNA.

Testicular FNA is a safe and reliable technique for the evaluation of the presence of mature cells of spermatogenesis in azoospermic men. It is successfully used in the treatment of azoospermia as an integrated part of ICSI. There are, however, many andrologists that are reluctant to fully support the widespread use of FNA, as there is still a need to increase our knowledge on spermatogenesis and to build a genetic database on male infertility with tissues obtained during open testicular biopsy. This database will then be used for in-depth genetic counseling in infertile men.

The studies comparing testicular FNA with open biopsy do not allow to safely draw definite conclusions. It is therefore sensible that each center follows a local protocol according to the individual needs and priorities. In the Unit of Reproductive Endocrinology of Aristotle University of Thessaloniki, there are certain clinical indications for the use of testicular FNA. The diagnostic accuracy of the method is very high, whereas an open biopsy follows as a second step in every case is considered necessary.

6. Clinical application of FNA

Testicular FNA biopsy holds a pivotal role in the diagnosis and the etiologic classification of a wide spectrum of andrological diseases. FNA biopsy concludes the investigations of men with INOA. This is a homogeneous group of infertile men with primary infertility, small testicular volume, elevated FSH, normal LH and testosterone levels, after exclusion of any known causes of infertility. A similar group of infertile men demonstrate all the INOA characteristics but show evidence of reduced androgen action; they are therefore diagnostically classified as Partial Androgen Deficiency of the Aging Male (PADAM). This group of men is characterized by clinical and laboratory partial hypogonadism: there is loss of libido, reduction of erections and low testosterone serum levels. Testicular FNA biopsy in these cases reveals severe hypospermatogenesis, maturation arrest or SCOS. Nevertheless, even in these cases of severe infertility, sperm retrieval is possible during biopsy. Spermatozoa can then be used in ART[30].

ART history is closely related to the testicular biopsy development. Palermo[31] and van Steirteghem[32] first described ICSI in 1992. FNA biopsy was used in ART for the first time in 1995 for sperm retrieval from azoospermic men[33]. Recent ART developments made fatherhood possible to azoospermic men, a group previously offered sperm donor as the only option. Azoospermia is generally classified as obstruc-

tive and non-obstructive. Ejaculation failure from a therapeuticpoint of view is included in the former group. In cases of ejaculation disorders, such as retrograde or ejaculation failure (meta-traumatic or psychogenic), sperm retrieval at FNA biopsy is up to 100%. Cytological or histological diagnosis is usually that of normal spermatogenesis or hypospermatogenesis[30].

Obstructive azoospermia can be caused by congenital agenesis of ejaculatory ducts (with or without cystic fibrosis), Young syndrome (epididymal obstruction with chronic tracheobronchitis), acquired epididymal or ejaculatory duct obstruction (usually post-infectious) and post-operative or post-failed anastomosis obstruction. Spermatozoa retrieval in these cases after testicular biopsy is up to 100% as well. In a similar way to the previously described group, cytological or histological diagnosis is usually normal spermatogenesis or mild hypospermatogenesis with evidence of partial arrest[35].

Non obstructive azoospermia is characterized by spermatic epithelium failure. This condition can be either idiopathic (INOA) or acquired, usually caused by cryptorchidism, orchitis, chromosomal abnormalities, chemotherapy or radiotherapy. Histological diagnosis varies from severe hypospermatogenesis and maturation arrest to SCOS[36,37]. Nevertheless, even in men with non obstructive azoospermia and SCOS or spermatogenesis arrest, normal spermatogenesis can be found during biopsy[38,39]. This is explained by the presence of testicular sites with focal spermatogenesis. Multiple biopsy sites increase the possibility of sperm retrieval from a testicular region with focal spermatogenesis. Spermatozoa retrieved from these sites can then be used in ICSI[40,41,42]. After a first positive FNA, the possibility of retrieving sperm in a subsequent FNA in non obstructive azoospermic men is almost 70%[43]. If hypospermatogenesis was diagnosed in the first FNA, the possibility is 91%, if SCOS 73%, if hyalinization of spermatic tubules 75%, but only 29% in cases where spermatogenesis arrest was found during the first testicular FNA.

According to Tournaye *et al,* ICSI success rates after open (TESE) or FNA biopsy is 44.0%, 45.7%, 67.8% and 62.5%, after spermatozoa retrieval from cases of SCOS, maturation arrest, hypospermatogenesis and normal spermatogenesis, respectively[44]. Pregnancy rates after embryo-transfer in the same groups of patients were 36.5%, 50.0%, 60.0%, and 43.5%, respectively[45].

In conclusion, FNA testicular biopsy can successfully assist in the sperm retrieval in almost every case of obstructive azoospermia. Pregnancy rates are satisfactory. In cases of ejaculation failure, FNA biopsy with ICSI are a feasible alternative to the ejaculation provocation with electrical stimulation. Finally, in almost 55% of men with non-obstructive azoospermia sperm can be successfully retrieved either with FNA or open testicular biopsy[45]. Pregnancy rates are satisfactory after successful embryo-transfer.

7. CONCLUSION

Testicular FNA biopsy is a significant laboratory technique for the investigation of selected cases of male infertility. Compared to open biopsy, FNA has a number of advantages; therefore it is already used as a diagnostic and therapeutic method in many andrology centers. FNA combined with the introduction of ICSI have revolutionized the management of male infertility in the recent years. Infertile men with severe spermatogenesis disorders can give birth to their own children, whereas only a few years ago the same group of men had only to choose between sperm donation and adoption.

Ο ρόλος της βιοψίας όρχεων με λεπτή βελόνη στην ανδρική υπογονιμότητα: Ανασκοπήσεις των ενδείξεων, των πλεονεκτημάτων και των κλινικών εφαρμογών

Θεμιστοκλής Μίκος, Δημήτριος Γ. Γουλής, Πάρις Πολυχρόνου, Γρηγόριος Γκριμπίζης, Ιωάννης Ν. Μπόντης, Ιωάννης Παπαδήμας

Μονάδα Ενδοκρινολογίας Αναπαραγωγής, Α΄ Μαιευτική και Γυναικολογική Κλινική
Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Γενικό Νοσοκομείο Παπαγεωργίου, Θεσσαλονίκη, Ελλάδα

ΠΕΡΙΛΗΨΗ: Η βιοψία όρχεων με λεπτή βελόνη (Fine Needle Aspiration - FNA) θεωρείται σήμερα αναπόσπαστο κομμάτι της διερεύνησης και της θεραπευτικής αντιμετώπισης της ανδρικής υπογονιμότητας, καθώς εκτός από την ιστολογική - κυτταρολογική διάγνωση δίνει τη δυνατότητα συλλογής σπερματοζωαρίων που στη συνέχεια θα χρησιμοποιηθούν στις τεχνικές μικρογονιμοποίησης (ICSI). Τα κύρια πλεονεκτήματα της FNA των όρχεων σε σύγκριση με την ανοικτή βιοψία είναι ότι αποτελεί ταχεία, χαμηλού κόστους, ατραυματική και λιγότερο παρεμβατική μέθοδο. Η χρήση της FNA σε συνδυασμό με την πρόσφατη εφαρμογή της ICSI έχουν επιφέρει επανάσταση στην αντιμετώπιση της ανδρικής υπογονιμότητας. Οι υπογόνιμοι άνδρες με διαταραχές στην σπερματογένεση έχουν πλέον τη δυνατότητα πατρότητας, ενώ κατά το παρελθόν κατέφευγαν σε μεθόδους όπως η ετερόλογη σπερματέγχυση ή η υιοθεσία.

Λέξεις Κλειδιά: FNA όρχεων, Ανδρική υπογονιμότητα, Αζωοσπερμία, ICSI.

REFERENCES

1. Chan PT, Schlegel PN. Nonobstructive azoospermia. Curr Opin Urol 2000, 10:617-624.
2. Wu M, Burstein DE. Fine needle aspiration. Cancer Invest 2004, 22:620-628.
3. Ford L, Rasgon BM, Hilsinger RL Jr, et al. Comparison of ThinPrep versus conventional smear cytopreparatory techniques for fine-needle aspiration specimens of head and neck masses. Otolaryngol Head Neck Surg 2002, 126:554-561.
4. Elliot D, Milla MR, Oghen K, et al. Expression of RBM in the nuclei of human germ cells is dependent on a critical region of the Y chromosome long arm. Proc Natl Acad Sci USA 1997, 94:3848-3853.
5. Craft I, Bennett V, Nicholson N. Fertilising ability of testicular spermatozoa. Lancet 1993, 342:864.
6. Schoysman R, Vanderzwalmen P, Nijs M, et al. Pregnancy after fertilization with human testicular spermatozoa. Lancet 1993, 342:1237.
7. Infertility, Report on Evaluation of the Azoospermic Male. An AUA Best Practice Policy and ASRM Practice Committee Report 2001, AUA & ASRM.
8. Obrant KO, Persson PS. Cytological study of the testis by aspiration biopsy in the evaluation of fertility. Urol Int 1965, 20:176-189.
9. Rosenlund B, Kvist U, Ploen L, Lundh Rozell B, Sjoblom P, and Hillensjo T. A comparison between open and percutaneous needle biopsies in men with azoospermia. Hum Reprod 1998, 13:1266-1271.
10. Gottschalk-Sabag S, Glick T, Bar-On E, Weiss DB. Testicular fine needle aspiration as a diagnostic method. Fertil Steril 1993, 59:1129-1131.
11. Sevastiadou P, Athanassiou E, Papanicolaou A, Grimbizis G, Mikos T, Goulis DG, Bontis I, Papadimas I. Testicular Fine Needle Aspiration cytology with ThinPrep method in the evaluation of male infertility. Andrologia 2004, 36:247 (abstract).
12. Meng MV, Cha I, Ljung BM, Turek PJ. Relationship between classic histological pattern and sperm findings on fine needle aspiration map in infertile men. Hum Reprod 2000, 15:1973-1977.
13. Foresta C, Varotto A. Assessment of testicular cytology by fine needle aspiration as a diagnostic parameter in the evaluation of the oligospermic subject. Fertil Steril 1992, 58:1028-1033.
14. Meng MV, Cha I, Ljung BM, Turek PJ. Testicular fine-needle aspiration in infertile men: correlation of cytologic pattern with biopsy histology. Am J Surg Pathol 2001, 25:71-79.
15. Aridogan I, Bayazit AY, Yaman M, Ersoz C, Doran S. Comparison of fine-needle aspiration and open biopsy of testis in sperm retrieval and histopathologic diagnosis. Andrologia 2003, 35:121-125.

16. Meng MV, Black LD, Cha I, Ljung BM, Pera RA, Turek PJ. Impaired spermatogenesis in men with congenital absence of the vas deferens. Hum Reprod 2001, 16:529-533.
17. Mallidis C, Baker HWG. Fine-needle tissue aspiration biopsy of the testis. Fertil Steril 1994, 61: 367-375.
18. Mahajan AD. The role of fine-needle aspiration cytology of the testis in the diagnostic evaluation of infertility. BJU Int 1999, 84:485-488.
19. Odabas O, Ugras S, Aydin S, Yilmaz S, Kemal Atilla M. Assessment of testicular cytology by fine needle aspiration and the imprint technique: are they reliable diagnostic modalities? Br J Urol 1997, 79: 445-448.
20. Gottschalk-Sabag S, Glick T, Weiss DB. Fine-needle aspiration of the testis and correlation with testicular open biopsy. Acta Cytol 1993, 37:67-72.
21. Harrington TG, Schauer D, Gilber B. Percutaneous testis biopsy: an alternative to open testicular biopsy in the evaluation of the infertile man. J Urol 1996, 156:1647-1651.
22. Schlegel PN, Su LM. Physiological consequences of testicular sperm extraction. Hum Reprod 1997, 12:1688-1692.
23. Jarrow JP. Clinical significance of intratesticular anatomy. J Urol 1991, 145:777-779.
24. Persson PS, Ahren C, Obrant KO. Aspiration biopsy smear of testis in azoospermia. Cytological versus histological examination. Scand J Urol Nephrol 1971, 5:22-26.
25. Hajdu SI, Melamed MR. Limitations of aspiration cytology in the diagnosis of primary neoplasms. Acta Cytol 1984, 28:337-345.
26. Highman WJ, Oliver RT. Diagnosis of metastases from testicular germ cell tumours using fine needle aspiration cytology. J Clin Pathol 1987, 40:1324-1333.
27. Craft I, Tsirigotis M, Courtauld E, Farrer-Brown G. Testicular needle aspiration as an alternative to biopsy for the assessment of spermatogenesis. Hum Reprod 1997, 12:1483-1487.
28. Ezeh UIO, Moore HDM, Cooke ID. A prospective study of multiple needle biopsies versus a single open biopsy for testicular sperm extraction in men with non-obstructive azoospermia. Hum Reprod 1998, 13:3075-3080.
29. Shufaro Y, Prus D, Laufer N, Simon A. Impact of repeated testicular fine needle aspirations (TEFNA) and testicular sperm extraction (TESE) on the microscopic morphology of the testis: an animal model. Hum Reprod 2002, 17:1795-1799.
30. Papadimas J, Goulis DG, Papanikolaou A, Grimbizis G, Tarlatzis BC, Bontis J. [Testicular Fine Needle Aspiration (FNA) biopsy: Useful tool in diagnosis and treatment of infertile man]. Anir 2004, 6:39-44.
31. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 1992, 340: 17-18.
32. Van Steirteghem AC, Liu J, Joris H, Nagy Z, Janssenswillen C, Tournaye H, et al. Higher success rate by intracytoplasmic sperm injection than by subzonal insemination. Report of a second series of 300 consecutive treatment cycles. Hum Reprod 1993, 8: 1055-1060.
33. Hovatta O, Moilanen J, von Smitten K, Reima I. Testicular needle biopsy, open biopsy, epididymal aspiration and intracytoplasmic sperm injection in obstructive azoospermia. Hum Reprod 1995, 10:2595-2599.
34. Tournaye H. Use of testicular sperm for the treatment of male infertility. Baillieres Clin Obstet Gynaecol 1997, 11:753-762.
35. Tournaye H. Surgical sperm recovery for intracytoplasmic sperm injection: which method is to be preferred? Hum Reprod 1999, 14(Suppl 1):71-81.
36. Lewin A, Reubinoff B, Porat-Katz A, Weiss D, Eisenberg V, Arbel R, et al. Testicular fine needle aspiration: the alternative method for sperm retrieval in non-obstructive azoospermia. Hum Reprod 1999, 14:1785-1790.
37. Turek PJ, Cha I, Ljung BM. Systematic fine-needle aspiration of the testis: correlation to biopsy and results of organ “mapping” for mature sperm in azoospermic men. Urology 1997, 49:743-748.
38. Steinberger E, Tjioe DY. Spermatogenesis in rat testes after experimental ischemia. Fertil Steril 1969, 20:639-649.
39. Levin HS. Testicular biopsy in the study of male infertility: its current usefulness, histologic techniques, and prospects for the future. Hum Pathol 1979, 10: 569-584.
40. Silber SJ, Van Steirteghem AC, Devroey P. Sertoli cell only revisited. Hum Reprod 1995, 10:1031-1032.
41. Friedler S, Raziel A, Strassburger D, Soffer Y, Komarovsky D, Ron-El R. Testicular sperm retrieval by percutaneous fine needle sperm aspiration compared with testicular sperm extraction by open biopsy in men with non-obstructive azoospermia. Hum Reprod 1997, 12:1488-1493.
42. Turek PJ, Givens CR, Schriock ED, Meng MV, Pedersen RA, Conaghan J. Testis sperm extraction and intracytoplasmic sperm injection guided by prior fine-needle aspiration mapping in patients with nonobstructive azoospermia. Fertil Steril 1999, 71:552-557.
43. Fasouliotis SJ, Safran A, Porat-Katz A, Simon A, Laufer N, Lewin A. A high predictive value of the first

testicular fine needle aspiration in patients with non-obstructive azoospermia for sperm recovery at the subsequent attempt. Hum Reprod 2002, 17:139-142.

44. Tournaye H, Liu J, Nagy PZ, Camus M, Goossens A, Silber S, et al. Correlation between testicular histology and outcome after intracytoplasmic sperm injection using testicular spermatozoa. Hum Reprod 1996, 11:127-132.

45. Zukerman Z, Orvieto R, Avrech OM, Weiss DB, Gottschalk-Sabag S, Bar-On E, et al. Is diagnostic testicular fine needle aspiration necessary in azoospermic men before sperm aspiration/extraction for intracytoplasmic sperm injection cycles? J Assist Reprod Genet 2000, 17:93-96.Magna faci bla commodit lortie magnism oluptatum vero cortion henismod ming ercip euguer si.